Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

Validation Report: D-Lactic Acid Assay Kit (cat. no. K-DATE)

1. Scope

Megazyme's D-Lactic Acid Assay Kit (K-DATE) is an enzymatic method used for the rapid and specific measurement and analysis of D-lactic acid in wine, beer, juice, milk, cheese, vinegar, meat and other food products. This D-Lactic Acid method was developed in-house and measures D-lactic acid in g/L. Methods based on this assay principle have been accepted by DIN, GOST, IDF, EEC, EN, ISO, OIV, IFU, AIJN and MEBAK.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by D-Lactic Acid Assay Kit (K-DATE).

3. Performance characteristics

The selectivity, working range, limit of detection, limit of quantification, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

This assay is specific for D-lactic acid.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding D-lactic acid to the sample in the initial extraction steps.

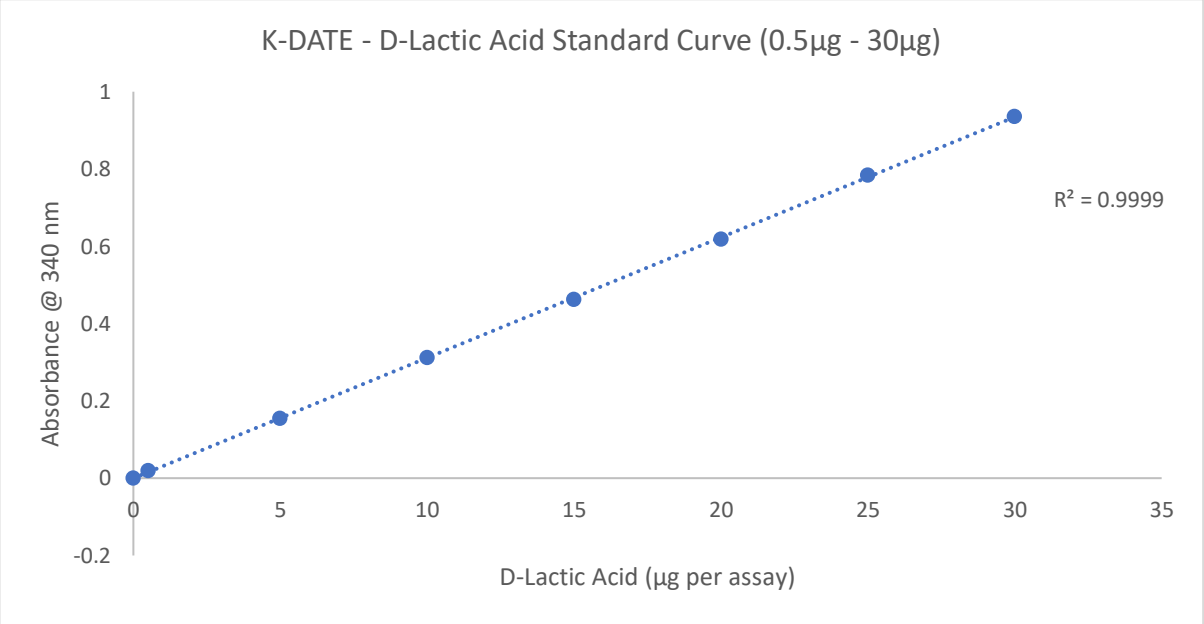
3.2. Working Range

Assay follows the D-Lactic Acid Assay Kit (K-DATE) standard procedure. 0.1 mL of D-lactic acid standard was used as a sample, with a range of concentrations (0.005-0.3 g/L) which corresponds to 0.5-30 µg of D-lactic acid per assay. Absorbance A2 was read after 5 min, at 340 nm and at 25°C as recommended in the procedure.

The working range is linear between 0.5-30 µg of D-lactic acid per assay.

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

D-Lactic Acid Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.5	0.019
5	0.155
10	0.312
15	0.462
20	0.618
25	0.784
30	0.936



Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.214 mg/L of D-lactic acid which is derived from an absorbance difference of 0.010 with a maximum sample volume of 1.5 mL.

The **calculated limit of detection (LOD)** and the **calculated limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the standard procedure of the D-Lactic Acid Assay (K-DATE).

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3 x σ of the blank sample solution absorbance and 10 x σ of the blank sample solution absorbance, respectively, where σ is the standard deviation of the absorbance values from 10 replicates.

- For D-Lactic Acid Assay Kit (K-DATE)

  LOD – For 1.5 mL of sample (maximum volume)
  D-Lactic Acid = 0.064 mg/L

  LOQ – For 1.5 mL of sample (maximum volume)
  D-Lactic Acid = 0.192 mg/L

* **Note**: The above detection limits are for samples as used in the assay, after any sample preparation, if required. The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the D-Lactic Acid Assay Kit (K-DATE) with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: ***b*(%) = x – xref / xref x 100.** The reference material for this purpose is D-lactic acid standard supplied with the D-Lactic Acid Assay Kit (K-DATE) at 0.15 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Lactic Acid	21	0.15	0.1507	0.44

3.5. Precision

This report details the reproducibility of the D-Lactic Acid Assay Kit (K-DATE), it is a measure of the variability in results on different occasions, by different analysts, over an extended period of time.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
D-Lactic Acid	21	0.15	0.1507	0.0021	1.38

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-Lactic Acid in various matrices. It is a novel method and is fully automatable for high throughput analysis of samples. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	D-Lactic Acid
Working range (µg in cuvette)	0.5 - 30
LOD (mg/L)	0.064
LOQ (mg/L)	0.192
Relative Bias *b*(%)	0.44
Reproducibility (%CV using kit standard)	1.38